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Nitro-, Azo- and Amino Derivatives of Ebselen: Synthesis, Structure and Cytoprotective Effects

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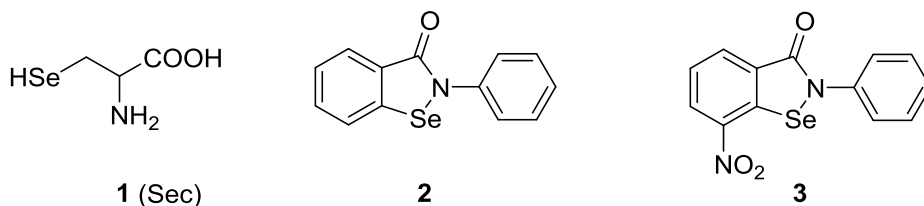
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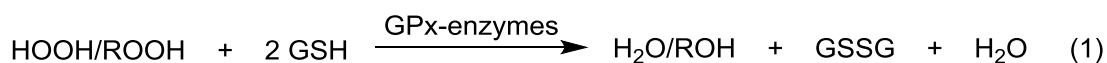
Abstract: Novel azo-bis-ebseleins **7** were prepared by reduction of 7-nitro-2-aryl-1,2-benzisoseleazol-3(2*H*)-ones **3** and **6** with sodium benzenetelluroate, NaTeC₆H₅, and by reaction of 2-bromo-3-nitrobenzamides with Na₂Se₂. The X-ray structure of **7b** showed that the molecule, due to strong intramolecular secondary Se···N interactions, is completely planar. Azo-compounds **7** upon further reaction with NaTeC₆H₅ were reductively cleaved to provide two equivalents of the corresponding aromatic amine. The weak Se–N bond was not stable enough to survive the reaction conditions and diselenides **8** were isolated after work-up. Whereas azo-bis-ebseleins **7** were poor mimics of the glutathione peroxidase (GPx)-enzymes, nitroebseleins **3**, **6** and **11b** and diselenides **8** were 3-6 fold more active than ebselen. Based on ⁷⁷Se NMR spectroscopy, a catalytic cycle for diselenide **8b**, involving aminoebseleins **14**, was proposed. As assessed by chemiluminescence measurements, the good GPx-mimics could reduce production of reactive oxygen species (ROS) in stimulated human mononuclear cells more efficiently than Trolox. No toxic effects of the compounds were seen in MC3T3-cells at 25 μM.

Introduction

Selenocysteine (Sec, **1**) is recognized as the 21st proteinogenic amino acid. It is an essential part of the active site of selenoproteins in humans and animals. So far, 23 selenoprotein families have been annotated.¹ The human selenoproteome consists of 25 selenoproteins.² Whereas some of these have a known structure and function (GPx, iodothyronine deiodinases, thioredoxin reductases)^{3,4} others are as yet insufficiently characterized. The substitution of selenium for sulfur in cysteine (Cys) has many



implications.⁵ At physiological pH, the more acidic selenol ($pK_a = 5.43$)⁶ is essentially deprotonated. Sec is therefore a more reactive nucleophile than Cys. Also, redox-cycling (oxidation/reduction) occurs more readily in Sec than in Cys. In addition to catalase, the GPx-enzymes are the most important hydroperoxide-decomposing enzymes in humans.⁷ Glutathione (GSH) is used as the stoichiometric reducing agent (eq 1). In the proposed catalytic cycle for the action of the GPx-enzymes selenium is present in the form of selenol, selenenic acid and selenosulfide.⁸



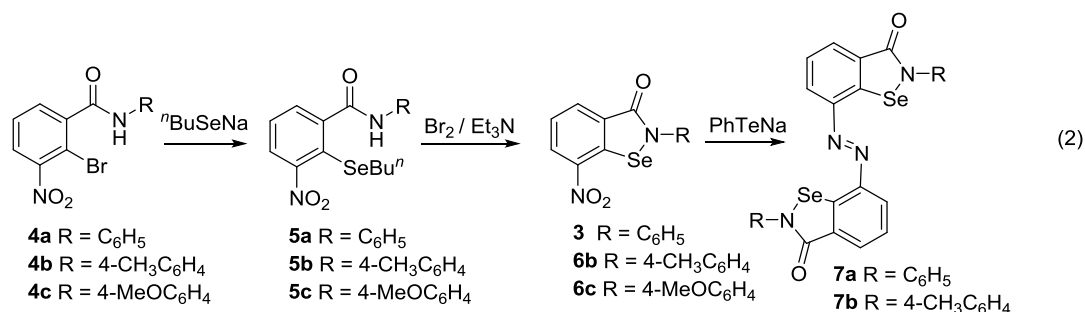
The reports on the existence of GPx triggered a search for small-molecule compounds that could mimic the action of the large enzyme. The benziselenazolone ebselen (**2**) was the first compound of this kind.⁹ Since the mid 1980s, the suitability of ebselen as a pharmaceutical agent has been extensively probed. Due to its GPx-activity, it has been found to reduce oxidative stress.¹⁰ It is/has been used in clinical trials for the prevention or treatment of cardiovascular diseases, arthritis, stroke and cancer.¹¹ Considered as a safe drug-like compound with a history of use in human clinical trials, ebselen has also been included in the National Institutes of Health Clinical Collection.¹² Ebselen has been subjected to numerous structural modifications in order to improve its GPx-activity.¹³ An early study by Parnham and co-workers showed that the nitro-derivative **3** of ebselen was 9-fold better than the parent as a catalyst for the glutathione-induced reduction of *t*-BuOOH.¹⁴ However, this finding has not been much explored and the reason for the rate enhancement has been attributed both to electronic¹⁵ and steric¹⁶ effects.

We thought it would be interesting to prepare benziselenazolones carrying a variety of *N*-substituents (nitro-, azo- and amino groups) in position 7. In the following we describe their synthesis and structure as well as their GPx-like activities and cytoprotective effects.

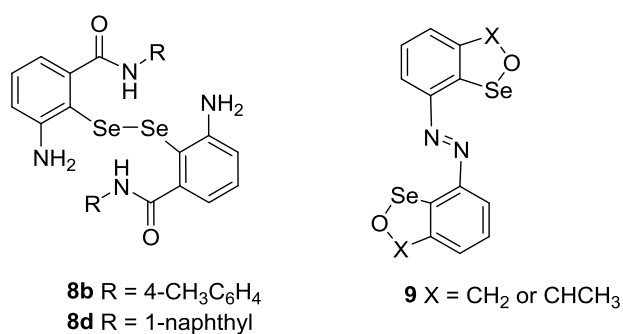
Results and Discussion

Synthesis: We envisaged obtaining azo- and amino derivatives of ebselen by reduction of the corresponding nitro compounds. Nitro-substituted ebselens were prepared using a slightly modified¹⁷ version of the procedure developed by Christians and co-workers.¹⁸ The required benzamides **4 a-c** (eq 2) for this reaction were obtained in high yields by addition of aniline, *para*-toluidine and *para*-anisidine, respectively, to 2-bromo-3-nitrobenzoyl chloride. Their conversion to

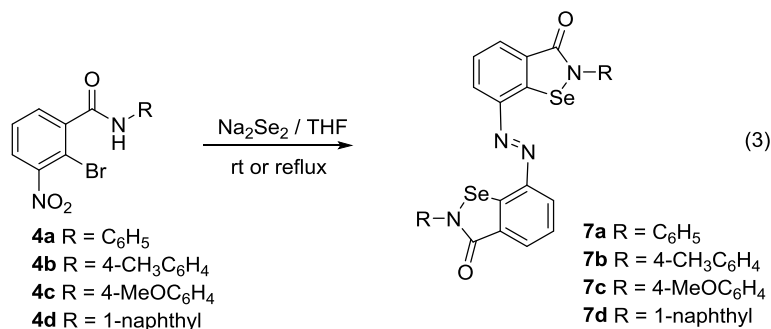
benzisoselenazolones **3/6b-c** involved aromatic nucleophilic substitution with $n\text{BuSeNa}$, generated *in situ* from Bu_2Se_2 and NaBH_4 in ethanol, followed by bromine induced cyclization.



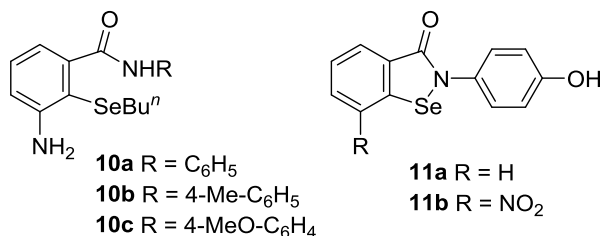
Reduction of the nitro group in the presence of the weak Se-N bond turned out to be difficult. Sodium benzenetelluroate, NaTePh ,¹⁹ generated in ethanol by sodium borohydride reduction of diphenyl ditelluride (Ph_2Te_2), was found to cleanly reduce compound **3** to azo-bis-ebseles derivative **7a** in 51% yield. However, in the case of **6b**, the corresponding azo-derivative **7b** was isolated as the minor product (9%) along with diselenide **8b** (16%). Obviously, in **8b**, the nitro group has been reduced all the way to an amine and the Se-N bond cleaved reductively. Oxidation of the resulting selenol then provided the diselenide product. Reduction of azo-compound **7b** with NaTePh produced diselenide **8b** as the only product (87%).



An alternative method for the preparation of azo-derivatives of ebselen was also tried. This is based on the finding that 2-bromo-3-nitrobenzyl alcohols, when reacted with disodium diselenide (Na_2Se_2) produced azo-derivatives **9** of 2-oxaselenaindane.²⁰ Reaction of *in situ*-prepared Na_2Se_2 with compounds **4a-d** at ambient temperature produced the corresponding azo-bis-ebseles **7a** (29%), **7b** (39%), **7c** (21% when heated at reflux) and **7d** (68%) (eq 3). Due to poor solubility, we were unable to characterize the methoxy derivative **7c**.



Further reaction of compound **7d** with NaTePh afforded diselenide **8d** in 52% yield. Under the similar reaction conditions, benzamides **5a-c**, carrying a butylseleno group in position 2, were cleanly reduced to the corresponding amines **10a-c** in high yields. We recently reported on the cytoprotective effects of the radical-trapping and hydroperoxide-decomposing ebselenol **11a**.²¹ Curious about the effects of an *ortho*-coordinating nitro group, compound **6c** was *O*-demethylated with 3 equivalents of BBr₃ in CH₂Cl₂ to afford the nitroebselenol **11b** in 88% yield.



Structure: The structures of azo-bis-ebesen derivative **7b** and diselenide **8b** were determined by X-ray crystallography. Dark black crystals of **7b** suitable for X-ray crystallographic analysis were obtained by slow evaporation of a CHCl₃-solution at -20 °C. The structure (Figure 1) shows strong intramolecular secondary Se···N interactions [Se1···N1 2.442(5) Å] notably shorter than the sum of the van der Waals radii of Se and N (3.45 Å).²² Normally, the bond length of a Se—N covalent bond is 1.87 Å. The strong secondary Se···N interactions causes a slight elongation of this bond (Se1—N2 = 1.940(3) Å). The coordination geometry is strongly distorted from a linear arrangement with the bond angle N2—Se1—N1 = 156.56(13)°, thus, indicating a T-shaped geometry around the Se atom. Because of these strong interactions, compound **7b** adopts a completely planar conformation. The crystal structure of **7b** showed π - π stacking interactions between the benzene ring with a centroid-centroid distance of 3.711 Å (Figure S3 in the Supporting Information).

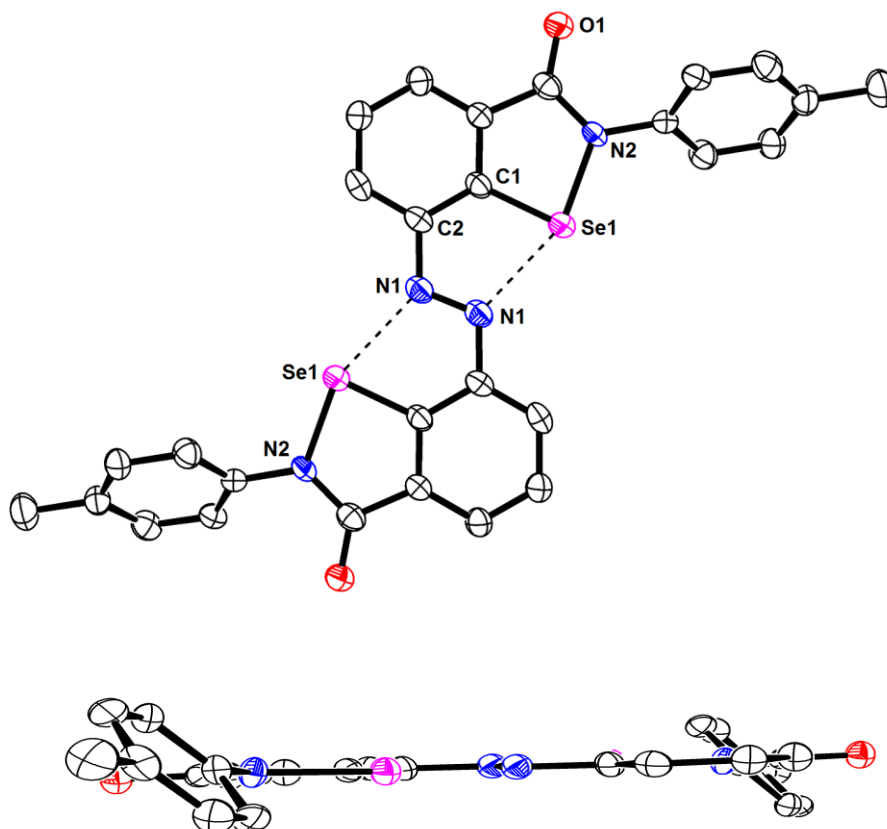


Figure 1. ORTEP diagram (up) top view and (down) side view of **7b**. Hydrogen atoms and solvated- CHCl_3 molecules are omitted for clarity. Thermal ellipsoids are set at 50% probability. Significant bond lengths [\AA] $\text{Se1}\cdots\text{N1}$ 2.442(5); $\text{Se1}-\text{C1}$ 1.851(4); $\text{Se1}-\text{N2}$ 1.940(3), and angles [$^\circ$] $\text{N2}-\text{Se1}-\text{C1}$ 84.04(16); $\text{N2}-\text{Se1}\cdots\text{N1}$ 156.56(13); $\text{C1}-\text{Se1}-\text{N1}$ 72.55(15).

Suitable orange crystals of diselenide **8b** were obtained for X-ray crystallographic analysis by slow evaporation of an ethyl acetate/pentane solution at room temperature. The structure (Figure 2) indicates a V-shaped geometry around the selenium atom with bond angles $\text{C1a}-\text{Se1}-\text{Se2}$ and $\text{C1b}-\text{Se2}-\text{Se1}$ of $104.26(5)^\circ$ and $103.65(5)^\circ$, respectively. The $\text{C1a}-\text{Se1}-\text{Se2}-\text{C1b}$ dihedral angle of $98.47(7)^\circ$ indicates a “transoid” conformation. As indicated in Figure 2, selenium is hydrogen bonded to the $-\text{NH}_2$ group in the ortho position. The $\text{Se}\cdots\text{H}$ distance of 2.734 \AA is significantly shorter than the sum of the van der Waals radii of selenium and hydrogen (3.10 \AA).²²

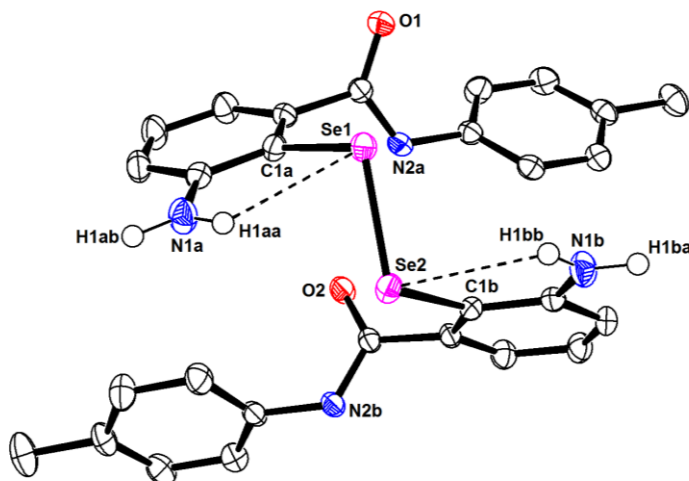


Figure 2. ORTEP diagram of **8b**. Hydrogen atoms are omitted for clarity. Thermal ellipsoids are set at 50% probability. Significant bond lengths [Å] Se1–C1a 1.9171(15); Se1–Se2 2.3602(2); Se2–C1b 1.9156(15), and angles [°] C1a–Se1–Se2 104.26(5); C1b–Se2–Se1 103.65(5); C1a–Se1–Se2–C1b 98.47(7).

Bonding: To find out more about the effect of intramolecular secondary Se \cdots N/O interactions on bonding and ^{77}Se NMR chemical shifts, density functional theory (DFT) calculations were carried out. The geometries of **6b**, **6c**, **7a**, **7b**, **7d**, and **11b** were fully optimized in the gas phase at the B3LYP/6-311+G(d) level of theory (for optimized geometries and coordinates see the Supporting Information). The optimized geometry of **7b** showed good agreement with the X-ray crystal structure. The structures of **7a** and **7d** were also found to be completely planar with strong Se \cdots N interactions. The second perturbation energies $E_{\text{Se}\cdots\text{N}}$ and $E_{\text{Se}\cdots\text{O}}$ shown in Table 1 were obtained by NBO analysis. The values reflect the effectiveness of orbital interactions between the low-lying $\sigma^*_{\text{Se-N}}$ and $n_{\text{N}/\text{O}}$. The magnitude of the $n_{\text{N}} \rightarrow \sigma^*_{\text{Se-N}}$ and $n_{\text{O}} \rightarrow \sigma^*_{\text{Se-N}}$ orbital interactions suggests a significant covalent interaction between the nitrogen lone pair and the low-lying antibonding orbital of the Se atom. The NBO results indicate that the Se \cdots N interactions were much stronger than the Se \cdots O interactions. The calculated ^{77}Se NMR chemical shifts were in good agreement with the experimental values.

Table 1. Data for **3**, **6b-c**, **7a-b**, **7d**, and **11b** obtained by DFT calculations at the B3LYP/6-311+G(d,p) level in the gas phase. The NBO analysis was calculated at the B3LYP/6-311+G(d,p) level using the B3LYP/6-311+G(d)-level-optimized geometries.

Second-order perturbation energy				NPA Charges	⁷⁷ Se NMR
Compounds	$r_{\text{Se}\cdots\text{O/N}}$ [Å]	$E_{\text{Se}\cdots\text{O/N}}$ (kcal/mol)	$r_{\text{Se-N}}$ [Å]	q_{Se}	(in ppm)
3 ^{17,23}	2.593 (2.573)	12.63	1.924(1.896)	+0.753	910 (924)
6b	2.598	12.35	1.922	+0.753	905 (929)
6c	2.604	12.00	1.921	+0.750	910 (932)
7a	2.532	17.32	1.946	+0.734	904 (931)
7b	2.537 (2.442)	16.89	1.944 (1.941)	+0.733	903 (927)
7d	2.539	16.57	1.943	+0.732	925 (946)
11b	2.601	12.22	1.922	+0.751	912 (923)

[a] The ⁷⁷Se NMR values are referenced to Me₂Se ($\delta = 0$). The experimental values are given in parentheses.

The topology of the electron density at the Se \cdots N/O bond critical point (BCP) was evaluated according to Bader's theory of atoms in molecules (AIM) using the AIM2000 software package (for molecular graphs, see Figure S6 in the Supporting Information). Parameters were calculated in order to support the NBO calculations in the form of electron density ($\rho_{\text{Se}\cdots\text{N/O}}$), Laplacian ($\nabla^2\rho_{\text{Se}\cdots\text{N/O}}$) and the total electron energy density ($H_{\text{Se}\cdots\text{N/O}}$) for the Se \cdots N/O interactions (Tables S19 and S20 in the Supporting Information). Whereas compounds **7a-b** and **7d** showed negative values for $H_{\text{Se}\cdots\text{N}}$, $H_{\text{Se}\cdots\text{O}}$ for compounds **6b-c** and **11b** were positive, suggesting weaker electrostatic interactions between Se and O atoms.

Glutathione Peroxidase-like Activity: The GPx-like activity of antioxidants **2-3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b** was assessed by the coupled reductase assay, using H₂O₂ as a substrate and GSH as a thiol cofactor in the presence of glutathione reductase (GR). GR serves to reduce oxidized glutathione (GSSG) formed by the action of the GPx-catalyst on H₂O₂/GSH, using β -nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. GPx-activities were assessed as the initial rates (v_0) for the consumption of NADPH by UV-spectroscopy at 340 nm during the initial 10 seconds of reaction. The values shown in Table 2 were corrected for the spontaneous oxidation of GSH by H₂O₂ ($24.3 \pm 1.2 \mu\text{M}\cdot\text{min}^{-1}$). Ebselen ($46.5 \pm 0.8 \mu\text{M}\cdot\text{min}^{-1}$) and **3** ($111.4 \pm 4.6 \mu\text{M}\cdot\text{min}^{-1}$) were included as bench-marks and references in the study. Nitroebseleins **6b**, **6c** and **11b** were found

to be nearly three times more active than ebselen (**2**), and slightly better than **3**. This enhanced activity of the nitro compounds can probably be ascribed to intramolecular secondary Se...O interactions. Such or similar effects are well described in the literature.^{14,17,20b,24} Azoebseleins **7a**, **7b** and **7d** turned out to be considerably poorer GPx-mimics than **2** and **3**. Interestingly, diselenide **8b** showed an activity ($271.6 \pm 5.7 \mu\text{M}\cdot\text{min}^{-1}$) almost six-fold larger than recorded for ebselen. The other diselenide **8d**, carrying a bulky naphthyl group, also showed good GPx-activity ($133.8 \pm 2.7 \mu\text{M}\cdot\text{min}^{-1}$).

Table 2. GPx-like activities of ebselen, **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b** as determined by the initial rate of NADPH consumption (v_0) in the presence of H₂O₂, GSH and glutathione reductase

Antioxidants	GPx-like activity, v_0 ($\mu\text{M}\cdot\text{min}^{-1}$)	Activity relative to ebselen
2 , ebselen	46.5 ± 0.8	1
3	111.4 ± 4.6	2.4
6b	135.2 ± 4.1	2.9
6c	137.4 ± 2.3	3.0
7a	29.5 ± 0.6	0.6
7b	2.3 ± 0.1	0.05
7d	9.7 ± 0.2	0.2
8b	271.6 ± 5.7	5.8
8d	133.8 ± 2.7	2.9
11b	123.9 ± 0.7	2.7

[a] Assay conditions: Phosphate buffer (100 mM), pH 7.5 with ethylenediaminetetraacetic acid (EDTA; 1 mM), GSH (1 mM), NADPH (0.2 mM), GR (1.3 unit·mL⁻¹), H₂O₂ (0.80 mM) and catalysts (20 μM). Stock solutions (2 mM) of catalysts **2**, **3** and **6a-b** were prepared in MeOH and stock solutions of catalysts **7**, **8** and **11b** were prepared in DMSO. Initial rates (v_0) were corrected for the spontaneous oxidation of GSH ($24.3 \pm 1.2 \mu\text{M}\cdot\text{min}^{-1}$). Errors correspond to \pm SD for triplicates.

Consumption of Hydrogen Peroxide: The performance of some of the best GPx-mimics **8b**, **8d** and **11b** (10 mol%) together with GSH, NADPH, GR and H₂O₂ in phosphate buffer was also followed for much longer time (250 min). As shown in Figure 3, a significant decrease in the absorbance of NADPH was seen during ca. 80 min. Thereafter, the consumption of NADPH corresponds more or less to the background reaction. Ebselen (**2**), included as a reference in Figure 3, could not match the activity of **8b**, **8d** and **11b**.

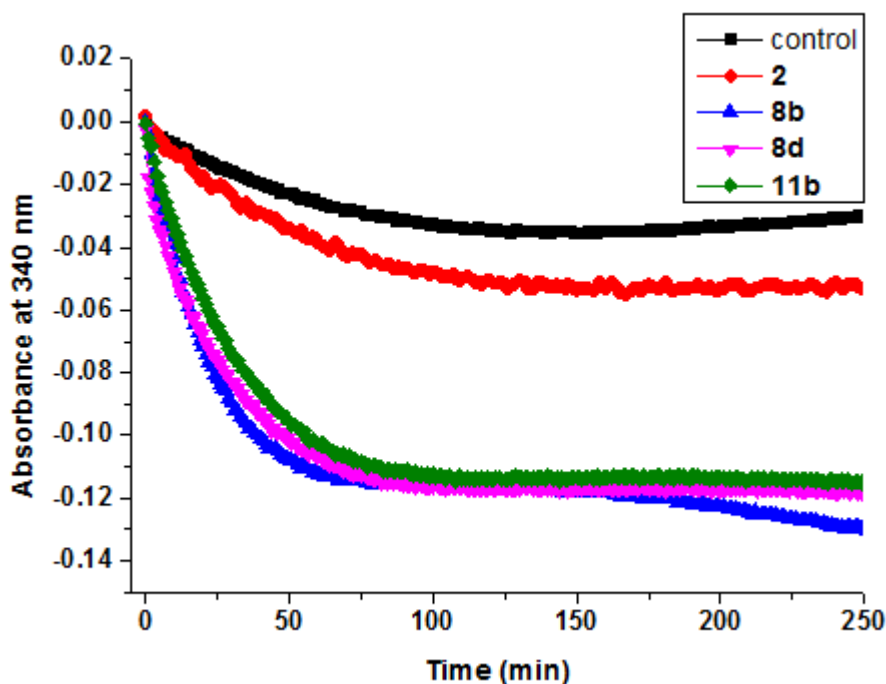
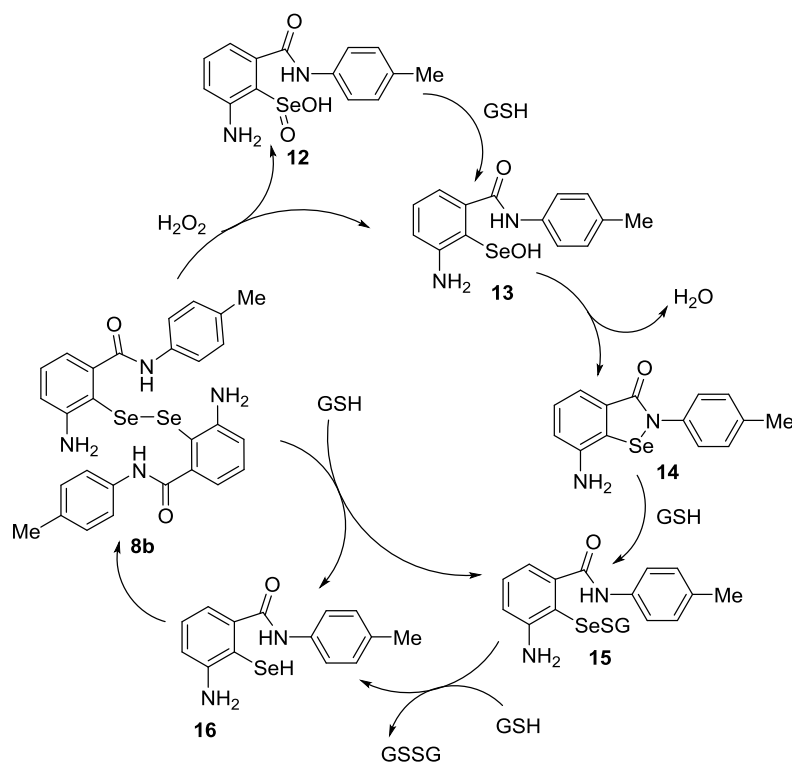


Figure 3. NADPH-consumption with time in the absence (control) and presence of catalysts **2** (ebselen), **8b**, **8d** and **11b**. A stock solution of catalyst **2** was prepared in MeOH and stock solutions of catalysts **8b**, **8d** and **11b** were prepared in DMSO. Assay conditions: reactions were carried out with phosphate buffer (100 mM), pH 7.5, with EDTA (1 mM), GSH (0.10 mM), NADPH (0.20 mM), GR (1.3 unit·mL⁻¹), H₂O₂ (20 μM) and catalyst (2 μM).

Mechanistic studies: In order to obtain some mechanistic insight, ⁷⁷Se NMR experiments in DMSO-d₆ were carried out with diselenide **8b** (δ = 369 ppm). Addition of 1 equivalent of H₂O₂ (Scheme 1) did not cause much of a change in the spectrum (see the ⁷⁷Se NMR spectrum in the Supporting Information). Upon addition of another equivalent of H₂O₂ to the above mixture, one new signal appeared at δ = 1156 ppm, corresponding to seleninic acid **12** (or a dehydrated product thereof - an *Se*-oxide of aminoebesen). Now, 1 equivalent of GSH was added to the mixture produced

containing unreacted **8b** and **12**. This caused the disappearance of the peak at $\delta = 1156$ and the appearance of two new peaks at $\delta = 935$ ppm and $\delta = 344$ ppm, corresponding to amino derivative **14** of ebselen and selenol **16**, respectively. Presumably, compound **14** is formed by cyclization of an intermediate selenenic acid **13**.^{17,24,25} Further addition of 2 equivalents of GSH to the mixture caused the disappearance of the peaks corresponding to **12**, **14** and **16** and only the peak at 369 ppm, corresponding to diselenide **8b**, remained in the ⁷⁷Se NMR spectrum. When GSH (1 equivalent) was added to a solution of **8b** in DMSO-d₆, the peak for **16** at $\delta = 346$ ppm appeared.



Scheme 1. Proposed mechanism for the reduction of H₂O₂ in the presence of GSH and diselenide **8b**

It is not surprising that the peak from selenol **16** is shifted only 25 ppm upfield from that of diselenide **8b**. This is due to strong intramolecular secondary Se···O interactions with the carbonyl group and additional substituent effects from the aromatic rings. A similar downfield chemical shift of 232 ppm for the corresponding selenol derived from ebselen was also accounted for in terms of strong Se···O interactions.^{24b} In none of the above experiments did we see a peak corresponding to selenosulfide **15**. It is probably a highly reactive species. We speculate that hydrogen bonding of Se to the ortho-amino group facilitates attack by thiol.

Cytoprotective effects: An overproduction of ROS/reactive nitrogen species (RNS) in biological systems has many deleterious effects. For example, oxidative damage of DNA is directly linked to

cardiovascular and neurodegenerative diseases.^{26,27} Since many of the novel antioxidants showed excellent hydroperoxide-decomposing activities, we decided to test their protective effects in cellular systems. Freshly isolated human mononuclear cells (MNC) were stimulated with phorbol myristate acetate (PMA) to produce ROS/RNS in the presence of antioxidants **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d**, **11b**, ebselen (**2**) and Trolox at 25 μ M. The total chemiluminescence (CL; extra- and intracellular) was then recorded in a luminol amplified assay. As shown in Figure 4, CL was significantly reduced for all compounds tested. Good GPx-mimics such as nitro compounds **6b**, **6c** and **11b** and diselenide **8b** all afforded better cytoprotection than Trolox. Nitroebselenol **11b** was by far the most potent protective agent.

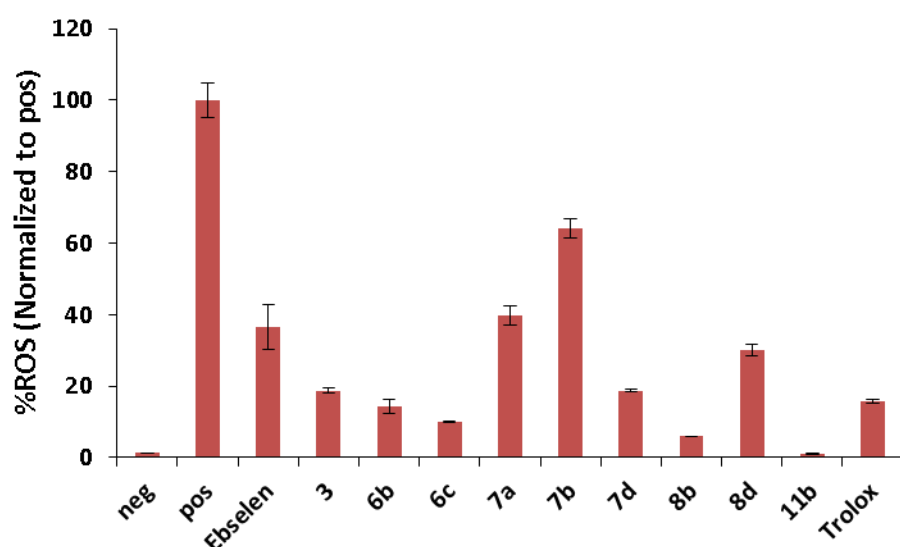


Figure 4. Chemiluminescence (normalized to positive control) monitored at $\lambda = 425$ nm in PMA-stimulated MNC-cells exposed to 25 μ M of antioxidants Ebselen (**2**), **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d**, **11b** and Trolox. N = 3 to 6 for each group from 4 independent experiments.

Cell Viability: In order to reveal any toxicity of antioxidants **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b**, MC3T3-cells (a preosteoblast cell line) were exposed to 25 μ M of compound and cell viability checked after 1 and 3 days by using the Alamar Blue assay (Figure 5). No toxic effects of the compounds were seen after 3 days.

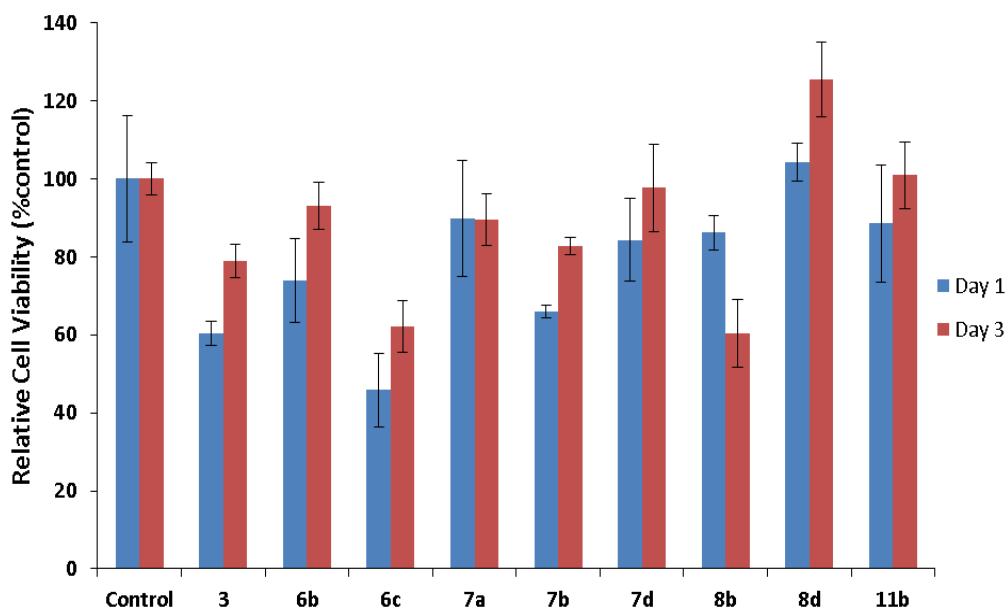


Figure 5. Relative cell viability of MC3T3 cells in the presence of 25 μM of antioxidants **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b** as determined by Alamar Blue measurements after 1 and 3 days at $\lambda_{\text{em}} = 590 \text{ nm}$.

Conclusion

In search for novel ebselen derivatives with improved antioxidative properties we have described straightforward syntheses of azo-bis-ebselens from nitroebseleins and 2-bromo-3-nitrobenzamides. Reduction of the azo-group to the corresponding amine was effected by NaTePh, but this transformation was accompanied by cleavage of the Se-N bond, resulting in formation of diaryl diselenides carrying both amino- and benzamide groups in the ortho positions. As revealed by X-ray crystallography and DFT-calculations, the intramolecular Se \cdots N-coordination in azo-bis-ebselens was much stronger than the corresponding Se \cdots O-interaction in nitroebseleins. This may be the reason for the poor GPx-activity of the azo-bis-ebselens. Both nitroebseleins **6** and diselenides **8** outperformed ebselen when it comes to catalysis of GSH-induced reduction of hydrogen peroxide. Considering their low toxicity and ability to inhibit ROS/RNS in stimulated human MNC-cells, we feel that compounds of this sort would be useful as models and tools in the development of novel treatments for diseases with a component of oxidative stress (cardiovascular diseases, stroke, Alzheimer's and Parkinson's diseases).

Experimental Section

2-Bromo-3-nitrobenzoic acid (98% purity) was purchased and used as such. ^1H and ^{13}C NMR spectra for all compounds prepared were recorded on 300 MHz (^1H : 300 MHz; ^{13}C : 75 MHz) and 400 MHz (^1H : 399.97 MHz; ^{13}C : 100 MHz) spectrometers, using the residual solvent peaks of CDCl_3 (^1H : δ 7.26; ^{13}C : δ 77.2), CD_3OD (^1H : δ 3.31; ^{13}C : δ 49.0) and DMSO-d_6 (^1H : δ 2.50; ^{13}C : δ 39.5), as indirect references to TMS ($\delta = 0$ ppm). ^{77}Se NMR spectra were recorded on 300 MHz (^{77}Se : 57 MHz) and 400 MHz (^{77}Se : 76 MHz) spectrometers with Ph_2Se_2 ($\delta = 460$ ppm) as an indirect reference to Me_2Se ($\delta = 0$ ppm). Flash column chromatography was performed using silica gel (0.04-0.06 mm). Melting points are uncorrected. The high resolution mass spectra (HRMS) were obtained using a time of flight (TOF) instrument equipped with electrospray ionization (ESI) and electron impact (EI^+) operating in the positive ion mode. Tetrahydrofuran (THF) was dried in a solvent purification system by passing it through an activated alumina column before use.

Typical procedure for benzamide formation: 2-Bromo-3-nitro-*N*-phenylbenzamide (4a). A mixture of 2-bromo-3-nitrobenzoic acid (1.0 g, 4.06 mmol) and thionyl chloride (5 mL) was refluxed for 3 h in the presence of a catalytic amount of *N,N*-dimethyl formamide (DMF). Excess thionyl chloride was then removed under reduced pressure. The residue was dissolved in dichloromethane (10 mL). Aniline (0.74 g, 8.13 mmol) in CH_2Cl_2 (15 mL) was added dropwise at room temperature. The resulting solution was then stirred for overnight. The reaction mixture was poured in water (10 mL) and extracted with dichloromethane. The separated organic layers were combined, dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using ethyl acetate/*n*-pentane (1:1) as an eluent to give the title product (1.13 g, 87%) as a solid. Physical and spectroscopic data were in good agreement with the literature.¹⁷ The ^1H and ^{13}C NMR spectra of **4a** have been included in the Supporting Information.

2-Bromo-3-nitro-*N-p*-tolylbenzamide (4b). White solid. Yield: 1.30 g (96%); Mp 166-168 °C; ^1H NMR (CDCl_3): δ 2.36 (s, 3H), 7.18 (d, $J = 8.2$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.53 (t, $J = 7.8$ Hz, 1H), 7.62 (br s, 1H), 7.71 (dd, $J = 1.7, 7.7$ Hz, 1H), 7.76 (dd, $J = 1.6, 7.9$ Hz, 1H); ^{13}C NMR (CDCl_3): δ 21.1, 111.6, 120.5, 126.2, 128.9, 129.9, 131.9, 134.6, 135.4, 141.5, 151.3, 164.2; HRMS (TOF MS ES^+) m/z calcd for $\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_3\text{Br}$ [$M+\text{H}$] $^+$: 335.0031; found: 335.0035.

2-Bromo-*N*-(4-methoxyphenyl)-3-nitrobenzamide (4c). The crude residue was purified by chromatography on silica gel with 60% ethyl acetate/pentane as an eluent to afford the title

compound as a white solid. Yield: 1.34 g (94%); Mp 180-182 °C; ¹H NMR (CD₃OD): δ 3.80 (s, 3H), 6.93 (dd, *J* = 2.2, 6.9 Hz, 2H), 7.57 (dd, *J* = 2.2, 6.9 Hz, 2H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.74 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.89 (dd, *J* = 1.7, 7.9 Hz, 1H); ¹³C NMR (CD₃OD): δ 55.9, 112.0, 115.1, 123.2, 126.6, 130.2, 132.3, 143.2, 152.8, 158.5, 166.9; HRMS (TOF MS ES⁺) *m/z* calcd for C₁₄H₁₁N₂O₄Br [*M*+H]⁺: 350.9980; found: 350.9975.

2-Bromo-*N*-(1-naphthyl)-3-nitrobenzamide (4d). The crude residue was washed with pentane to obtain the title compound of sufficient purity. Yield: 0.89 g (59%); Mp 243-246 °C; ¹H NMR (DMSO-*d*₆): δ 7.56-7.59 (several peaks, 3H), 7.80 (m, 3H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.97-8.00 (several peaks, 2H), 8.10 (dd, *J* = 1.2, 6.8 Hz, 1H), 8.20 (t, *J* = 7.2 Hz, 1H), 10.75 (s, 1H); ¹³C NMR (DMSO-*d*₆): δ 110.5, 122.6, 122.9, 125.3, 125.6, 126.2, 126.3, 126.4, 128.2 (2C), 129.5, 131.8, 132.7, 133.8, 141.9, 150.9, 165.6; HRMS (TOF MS EI⁺) *m/z* calcd for C₁₇H₁₁N₂O₃Br [*M*]⁺: 369.9953; found: 369.9959.

Typical procedure for the introduction of a butylseleno group by nucleophilic aromatic substitution: 2-Butylseleno-3-nitro-*N*-phenylbenzamide (5a). Benzamide **4a** (0.75 g, 2.33 mmol) was added to an *in situ*-prepared solution of BuSeNa (4 equivalents) in EtOH (30 mL) at 0 °C under inert atmosphere. The mixture was stirred at room temperature for 2 h and then warmed at 50 °C for 20 h. The solvent was removed under reduced pressure and the residue dissolved in CHCl₃ and washed with water. After drying over anhydrous Na₂SO₄, evaporation of the solvent and column chromatography using 20% ethyl acetate/pentane as eluent, the pure title compound (0.51 g, 55%) was isolated. Physical and spectroscopic data were in good agreement with the literature.¹⁷ The ¹H, ¹³C and ⁷⁷Se NMR spectra of **5a** have been included in the Supporting Information.

2-Butylseleno-3-nitro-*N-p*-tolylbenzamide (5b). Orange liquid (semi-solid). Solidified in the freezer. Yield: 0.59 g (67%); Mp 69-73 °C; ¹H NMR (CDCl₃): δ 0.78 (t, *J* = 7.3 Hz, 3H), 1.22-1.29 (several peaks, 2H), 1.45-1.55 (several peaks, 2H), 2.36 (s, 3H), 2.87 (t, *J* = 7.5 Hz, 2H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.49-7.55 (several peaks, 3H), 7.83 (dd, *J* = 1.3, 7.9 Hz, 1H), 7.91 (dd, *J* = 1.4, 7.6 Hz, 1H), 8.24 (br s, 1H); ¹³C NMR (CDCl₃): δ 13.5, 21.1, 22.7, 31.1, 31.9, 120.0, 122.4, 125.5, 128.9, 129.9, 133.4, 135.0, 135.1, 142.9, 155.1, 165.2; ⁷⁷Se NMR (CDCl₃): δ 283; HRMS (TOF MS ES⁺) *m/z* calcd for C₁₈H₂₀N₂O₃Se [*M*+H]⁺: 393.0717; found: 393.0721.

2-Butylseleno-*N*-(4-methoxyphenyl)-3-nitrobenzamide (5c). Yellow solid. Yield: 0.50 g (56%); Mp 94-97 °C; ¹H NMR (CDCl₃): δ 0.78 (t, *J* = 7.3 Hz, 3H), 1.26 (m, 2H), 1.51 (m, 2H), 2.88 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 6.93 (dd, *J* = 2.2, 7.7 Hz, 2H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.57 (dd, *J* = 2.2, 5.4 Hz, 2H), 7.82 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.90 (dd, *J* = 1.5, 7.6 Hz, 1H), 8.22 (br s, 1H); ¹³C NMR (CDCl₃): δ 13.5, 22.7, 31.0, 31.9, 55.6, 114.5, 121.7, 122.3, 125.3, 128.8, 130.7, 133.1, 143.0, 155.0, 157.0, 165.2; ⁷⁷Se NMR (CDCl₃): δ 283; HRMS (TOF MS ES⁺) *m/z* calcd for C₁₈H₂₀N₂O₄Se [*M*+H]⁺: 409.0667; found: 409.0663.

Typical procedure for the synthesis of nitroebesen analogues: 7-Nitro-2-phenyl-1,2-benzisoselenazol-3(2*H*)-one (3). To a stirred solution of selenide **5a** (0.72 g, 1.84 mmol) in dry CHCl₃ (10 mL) was added Br₂ (0.10 ml, 1.84 mmol) in dry CHCl₃ (5 mL) containing Et₃N (0.257 ml, 1.84 mmol) at 0 °C under an inert atmosphere. The reaction mixture was then stirred for 3 h at room temperature and water (10 mL) was added. The separated organic layer was dried over anhydrous Na₂SO₄. Removal of the solvent and purification of the residue by silica gel column chromatography (elution with 25% ethyl acetate/*n*-pentane) afforded the title compound (0.58 g, 95%) as a red solid. Physical and spectroscopic data were in good agreement with the literature.¹⁷ The ¹H, ¹³C and ⁷⁷Se NMR spectra of **3** have been included in the Supporting Information.

7-Nitro-2-*p*-tolyl-1,2-benzisoselenazol-3(2*H*)-one (6b). Yield: 0.50 g (82%); Mp 184 °C; ¹H NMR (CDCl₃): δ 2.39 (s, 3H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.72 (t, *J* = 7.8 Hz, 1H), 8.46 (dd, *J* = 0.9, 7.5 Hz, 1H), 8.57 (dd, *J* = 0.9, 8.1 Hz, 1H); ¹³C NMR (CDCl₃): δ 21.3, 125.2, 127.6, 127.9, 130.3, 131.6, 135.3, 135.8, 136.6, 137.4, 142.2, 164.0; ⁷⁷Se NMR (CDCl₃): δ 929; HRMS (TOF MS ES⁺) *m/z* calcd for C₁₄H₁₀N₂O₃Se [*M*+H]⁺: 334.9935; found: 334.9943.

2-(*p*-Methoxyphenyl)-7-nitro-1,2-benzisoselenazol-3(2*H*)-one (6c). Purification with 40% ethyl acetate/*n*-pentane afforded the pure title compound as a red solid. Yield: 67%; Mp 206-209 °C; ¹H NMR (CDCl₃): δ 3.86 (s, 3H), 6.99 (d, *J* = 9.0 Hz, 2H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.73 (t, *J* = 7.6 Hz, 1H), 8.47 (d, *J* = 7.6 Hz, 1H), 8.58 (d, *J* = 8.1 Hz, 1H); ¹³C NMR (CDCl₃): δ 55.8, 114.9, 127.1, 127.6, 127.9, 131.0, 131.4, 135.3, 136.6, 142.2, 158.9, 164.2; ⁷⁷Se NMR (CDCl₃): δ 932; HRMS (TOF MS ES⁺) *m/z* calcd for C₁₄H₁₀N₂O₄Se [*M*+H]⁺: 350.9884; found: 350.9876.

Typical procedure A for the preparation of azo-bis-ebesen compounds: Bis-[2-phenyl-1,2-benzisoselenazol-3-(2*H*)-one-7-yl]diazene (7a). Nitroebesen **3** (275 mg, 0.86 mmol) was added to

an *in situ* prepared colourless solution of PhTeNa (1.72 mmol) from the reaction of Ph₂Te₂ (352 mg, 0.86 mmol) and NaBH₄ (65 mg, 1.73 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at reflux for 20 h, allowed to cool, poured into water and extracted with CHCl₃. The separated CHCl₃-extract was dried over anhydrous Na₂SO₄, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel. The column was first eluted with pentane/ethyl acetate (50:50) then with CHCl₃ and finally with CHCl₃/MeOH (98:2) to give the title compound as a dark brown solid. Yield: 0.125 g (51%); Mp >320 °C; ¹H NMR (CDCl₃): δ 7.32 (t, *J* = 7.2 Hz, 2H), 7.49 (t, *J* = 7.2 Hz, 4H), 7.66 (dd, *J* = 8.0 Hz, 4H), 7.80 (t, *J* = 7.6 Hz, 2H), 8.40 (d, *J* = 7.2 Hz, 2H), 8.61 (d, *J* = 7.6 Hz, 2H); ¹³C NMR (CDCl₃): δ 125.3, 126.7, 128.3, 129.6, 130.2, 131.7, 131.8, 132.1, 139.4, 144.5, 164.0; ⁷⁷Se NMR (CDCl₃): δ 931; HRMS (MALDI) *m/z* calcd for C₂₆H₁₆N₄O₂Se₂ [*M*+H]⁺: 576.9682; found: 576.9689.

Bis-[2-*p*-tolyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7b). Heating at reflux for 40 h. Purification by column chromatography using 4% MeOH/CHCl₃. Yield: 30 mg (9%); Mp >320 °C; ¹H NMR (CDCl₃): δ 2.41 (s, 6H), 7.29 (d, *J* = 7.6 Hz, 4H), 7.52 (d, *J* = 8.0 Hz, 4H), 7.78 (t, *J* = 7.2 Hz, 2H), 8.38 (d, *J* = 7.6 Hz, 2H), 8.57 (d, *J* = 7.6 Hz, 2H); ¹³C NMR (CDCl₃): δ 21.3, 125.4, 128.2, 130.2, 131.2, 131.8, 132.1, 136.7 (2C), 144.6, 164.1; ⁷⁷Se NMR (CDCl₃): δ 927; HRMS (TOF MS ES⁺) *m/z* calcd for C₂₈H₂₀N₄O₂Se₂ [*M*+H]⁺: 604.9995; found: 604.9970.

Typical procedure B for the preparation of azo-bis-ebsele compounds: Bis-[2-phenyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7a). To a brown suspension of *in situ* prepared Na₂Se₂ (1.24 mmol) in dry THF (20 mL) under an inert atmosphere was slowly added a solution of benzamide **4a** (0.20 g, 0.62 mmol) in THF (10 mL) at room temperature. After heating at reflux for 5h and cooling to room temperature, water (20 mL) was added and stirring was continued for 10 min. Following extraction with CHCl₃, the combined organic layers were dried over anhydrous Na₂SO₄. Removal of the solvent and purification of the residue by silica gel column chromatography, eluting first with pentane/ethyl acetate (50:50) then with CHCl₃ and finally with CHCl₃/MeOH (98:2), afforded compound **7a** (62 mg, 35%). Similarly, compound **7b** (340 mg, 36%) was prepared from **4b** (1.00 g, 2.98 mmol).

Bis-[1-naphthyl-1,2-benzisoselenzol-3-(2*H*)-one-7-yl]diazene (7d). The solution of **4d** and Na₂Se₂ was stirred for 8 h at room temperature before workup. Purification by column chromatography using 5% MeOH/CHCl₃ as an eluent afforded the title compound (68%) as a dark brown solid. Mp

>320 °C; ¹H NMR (CDCl₃): δ 7.49-7.61 (several peaks, 8H), 7.77 (t, *J* = 8.0 Hz, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.95 (t, *J* = 6.0 Hz, 4H), 8.42 (d, *J* = 7.6 Hz, 2H), 8.53 (d, *J* = 8.0 Hz, 2H); ¹³C NMR (CDCl₃): δ 123.5, 125.8, 126.8 (2C), 127.1, 128.3, 128.7, 129.1, 130.8, 130.9, 131.4, 131.7, 132.4, 134.8, 135.0, 144.5, 165.3; ⁷⁷Se NMR (CDCl₃): δ 946; HRMS (TOF MS EI⁺) *m/z* calcd for C₃₄H₂₀N₄O₂Se₂ [M]⁺: 675.9917; found: 675.9913.

Typical procedure for the synthesis of diselenides: Bis[3-amino-*N*-(1-naphthyl)benzamide-2-yl] diselenide (8d). The azo-bis-ebsele **7d** (50 mg, 0.074 mmol) was added to an *in situ*-prepared colorless solution of NaTePh (0.592 mmol) prepared from Ph₂Te₂ (61 mg, 0.148 mmol) and NaBH₄ (11 mg, 0.296 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at 80 °C for 1 h. and allowed to cool to room temperature. After addition of water, the mixture was extracted with ethyl acetate and dried over anhydrous MgSO₄. Evaporation of the solvent and purification by column chromatography, using ethyl acetate as an eluent afforded the title compound (25 mg, 52%) as a yellow solid. Mp 236-240 °C; ¹H NMR (DMSO-*d*₆): δ 5.63 (s, 4H), 6.45 (d, *J* = 7.1 Hz, 2H), 6.80 (d, *J* = 7.8 Hz, 2H), 6.90 (t, *J* = 7.5 Hz, 2H), 7.25 (d, *J* = 7.0 Hz, 2H), 7.25-7.52 (several peaks, 6H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.97 (d, *J* = 7.9 Hz, 2H), 10.27 (s, 2H); ¹³C NMR (DMSO-*d*₆): δ 110.1, 114.3, 114.8, 122.6, 123.0, 125.3, 125.5, 125.7, 125.8, 127.8, 128.4, 130.3, 133.1, 133.5, 144.5, 150.9, 169.2; ⁷⁷Se NMR (DMSO-*d*₆): δ 364; HRMS (TOF MS ES⁺) *m/z* calcd for C₃₄H₂₆N₄O₂Se₂ [M+Na]⁺: 705.0286; found: 705.0304.

Bis[3-Amino-*N*-(*p*-tolyl)benzamide-2-yl] diselenide (8b). Orange solid. Yield: 44 mg (87%); Mp 219-221 °C; ¹H NMR (DMSO-*d*₆): δ 2.25 (s, 6H), 5.46 (s, 4H), 6.60 (d, *J* = 7.0 Hz, 2H), 6.79 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.2 Hz, 4H), 7.10 (t, *J* = 7.8 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 4H), 10.08 (s, 2H); ¹³C NMR (DMSO-*d*₆): δ 20.5, 110.2, 114.6, 115.0, 120.0, 128.8, 130.4, 132.4, 136.3, 144.7, 150.8, 167.3; ⁷⁷Se NMR (DMSO-*d*₆): δ 368; HRMS (TOF MS ES⁺) *m/z* calcd for C₂₈H₂₆N₄O₂Se₂ [M+H]⁺: 611.0464; found: 611.0475.

Diselenide **8b** was also isolated in 16% yield when **6b** was allowed to react with NaTePh.

Typical procedure for reduction of 2-butylseleno-3-nitrobenzamides: 3-Amino-2-butylseleno-*N*-phenyl benzamide (10a). To *in situ*-prepared NaTePh (0.96 mmol) from the reaction of Ph₂Te₂ (192 mg, 0.48 mmol) and NaBH₄ (35 mg, 0.94 mmol) in EtOH (10 mL) was added **5a** (96 mg 0.24 mmol) at room temperature under nitrogen. The mixture was then heated at reflux for overnight. After cooling to room temperature the solvent was evaporated and the residue dissolved in

dichloromethane. After treatment with water the organic layer was dried over anhydrous MgSO₄ and the solvent removed under reduced pressure. Chromatographic purification, eluting with 40% ethyl acetate/pentane, afforded the title compound (70 mg, 96%) as a white solid; Mp 76-78 °C; ¹H NMR (CDCl₃): δ 0.83 (t, *J* = 7.5 Hz, 3H), 1.33 (m, 2H), 1.58 (m, 2H), 2.81 (t, *J* = 7.5 Hz, 2H), 4.62 (s, 2H), 6.83 (d, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 6.9 Hz, 1H), 7.12-7.22 (several peaks, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.8 Hz, 2H), 7.79 (s, 1H); ¹³C NMR (CDCl₃): δ 13.6, 23.0, 29.3, 32.6, 110.4, 115.8, 117.6, 120.1, 124.5, 129.2, 130.1, 138.2, 144.4, 149.6, 168.0; ⁷⁷Se NMR (CDCl₃): δ 145; HRMS (TOF MS EI⁺) *m/z* calcd for C₁₇H₂₀N₂OSe [M]⁺: 348.0741; found: 348.0742.

3-Amino-2-butylseleno-*N*-(*p*-tolyl)benzamide (10b). Yield: 82%; Mp 88-90 °C; ¹H NMR (CD₃OD): δ 0.84 (t, *J* = 7.0 Hz, 3H), 1.35 (m 2H), 1.58 (m 2H), 2.32 (s, 3H), 2.77 (t, *J* = 7.4 Hz, 2H), 6.74 (dd, *J* = 1.2, 7.5 Hz, 1H), 6.86 (dd, *J* = 1.2, 8.2 Hz, 1H), 7.32-7.18 (several peaks, 3H), 7.52 (d, *J* = 8.3 Hz, 2H); ¹³C NMR (CD₃OD): δ 13.9, 21.0, 23.9, 28.7, 33.6, 111.6, 116.2, 116.7, 121.7, 130.2, 130.9, 135.1, 137.3, 146.7, 151.6, 171.5; ⁷⁷Se NMR (CD₃OD): δ 137; HRMS (TOF MS EI⁺) *m/z* calcd for C₁₈H₂₂N₂OSe [M]⁺: 362.0897; found: 362.0901.

3-Amino-2-(butylseleno)-*N*-(4-methoxyphenyl)benzamide (10c). Yield: 79%; Mp 100-104 °C; ¹H NMR (DMSO-*d*₆): δ 0.79 (t, *J* = 7.4 Hz, 3H), 1.25-1.31 (several peaks, 2H), 1.45-1.51 (several peaks, 2H), 2.71 (t, *J* = 7.4 Hz, 2H), 3.73 (s, 3H), 5.52 (br s, 2H), 6.58 (d, *J* = 7.0 Hz, 1H), 6.81 (d, *J* = 8.3 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 2H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.61 (d, *J* = 8.6 Hz, 2H), 9.99 (s, 1H); ¹³C NMR (DMSO-*d*₆): δ 13.4, 22.3, 26.9, 31.9, 55.2, 108.6, 113.7, 114.1, 114.4, 120.9, 129.4, 132.7, 145.9, 150.3, 155.2, 167.7; ⁷⁷Se NMR (DMSO-*d*₆): δ 144; HRMS (TOF MS EI⁺) *m/z* calcd for C₁₈H₂₂N₂O₂Se [M]⁺: 378.0846; found: 378.0844.

2-(4-Hydroxyphenyl)-7-nitrobenziselenazol-3(2*H*)-one (11b). Solution of BBr₃ (0.86 mmol, 0.86 mL) was added into a stirred solution of **6c** (0.27 mmol, 0.10 g) in dry CH₂Cl₂ (10 mL) at -78 °C under an inert atmosphere. Stirring was then continued for overnight at room temperature. The reaction mixture was diluted with dichloromethane and poured into a solution of brine. The separated organic layer was dried over anhydrous MgSO₄. The solvent was removed under reduced pressure to afford a red solid which was washed with diethyl ether and dried under vacuum to give the pure title compound. (85 mg, 88%); Mp 263-265 °C; ¹H NMR (DMSO-*d*₆): δ 6.86 (d, *J* = 9.0 Hz, 2H), 7.41 (d, *J* = 9.0 Hz, 2H), 7.86 (t, *J* = 7.9 Hz, 1H), 8.38 (d, *J* = 7.4 Hz, 1H), 8.71 (d, *J* = 8.2 Hz, 1H), 9.77 (s, 1H); ¹³C NMR (DMSO-*d*₆): δ 115.9, 127.1, 128.3, 128.8, 129.3, 130.3, 134.8, 135.8, 142.0, 156.6,

163.6; ^{77}Se NMR (DMSO- d_6): δ 923; HRMS (TOF MS EI^+) m/z calcd for $\text{C}_{13}\text{H}_8\text{N}_2\text{O}_4\text{Se}$ [M] $^+$: 335.9649; found: 335.9642.

X-ray Crystallographic Analysis. X-ray crystallographic studies for obtaining the structures of compounds **7b** and **8b** were carried out using graphite-monochromatized Mo $K\alpha$ radiation ($\lambda = 0.7107 \text{ \AA}$). The structures were solved by direct methods (SHELXS-2013) and refined by a full-matrix least-squares procedure on F^2 for all reflections using SHELXL-2013 software.²⁸ Hydrogen atoms were localized by geometrical means. A riding model was chosen for refinement. The isotropic thermal parameters of hydrogen atoms were fixed at 1.5 times and 1.2 times $U(\text{eq})$ of the corresponding carbon atoms for sp^3 C-H and sp^2 C-H bonds, respectively. Crystallographic data for the structures reported in this paper, containing supplementary crystallographic data for this paper, have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications. CCDC 1047473 (for compound **7b**), and CCDC 1047474 (for compound **8b**). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Crystal data for 7b. $\text{C}_{30}\text{H}_{22}\text{Cl}_6\text{N}_4\text{O}_2\text{Se}_2$, $M_r = 841.14$, Monoclinic, space group $C2/c$, $a = 22.883(7) \text{ \AA}$, $b = 12.0629(12) \text{ \AA}$, $c = 15.901(5) \text{ \AA}$, $\alpha = \gamma = 90^\circ$, $\beta = 132.43(5)^\circ$, $V = 3239.5(15) \text{ \AA}^3$, $\lambda = 0.71073 \text{ \AA}$, $Z = 4$, $T = 123(2) \text{ K}$, $\rho_{\text{calcd}} = 1.725 \text{ Mg/m}^3$, $\text{GOF} = 1.049$, $R1 = 0.0707$, $wR2 = 0.1422$ [$I > 2\sigma(I)$]; $R1 = 0.1291$, $wR2 = 0.1754$ (all data). Of the 18727 reflections that were collected, 6174 were unique ($R_{\text{int}} = 0.1199$).

Crystal data for 8b. $\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_2\text{Se}_2$, $M_r = 608.45$, Monoclinic, space group $P 1 2 / c 1$, $a = 8.9772(2) \text{ \AA}$, $b = 26.5501(5) \text{ \AA}$, $c = 10.9101(3) \text{ \AA}$, $\alpha = \gamma = 90^\circ$, $\beta = 96.200(2)^\circ$, $V = 2585.16(10) \text{ \AA}^3$, $\lambda = 0.71073 \text{ \AA}$, $Z = 4$, $T = 123(2) \text{ K}$, $\rho_{\text{calcd}} = 1.563 \text{ Mg/m}^3$, $\text{GOF} = 1.069$, $R1 = 0.0543$, $wR2 = 0.0813$ [$I > 2\sigma(I)$]; $R1 = 0.0981$, $wR2 = 0.0921$ (all data). Of the 59472 reflections that were collected, 16750 were unique ($R_{\text{int}} = 0.0599$).

Computational details. Computational calculations for compounds **6b-c**, **7a-b**, **7d**, **11b** were executed by using the Gaussian 09 suite of quantum chemical programs.²⁹ The hybrid B3LYP exchange correlation functional was implemented for density functional theory (DFT) calculations.³⁰ The geometry optimizations were carried out at the B3LYP level of DFT by using the 6-311+G(d) basis sets. The quantifications of orbital interaction were done by natural bond orbital (NBO) analysis at B3LYP/6-311+G(d,p) level.³¹ The ^{77}Se NMR calculations were performed at B3LYP/6-311+G(d,p) level on B3LYP/6-311+G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method (referenced with respect to the peak of Me_2Se).³² Atoms in molecules

(AIM)³³ calculations have also been used to confirm distinct bond critical point between the two interacting atoms.

Coupled Reductase Assay. The glutathione peroxidase-like activity of compounds prepared was determined by UV-spectroscopy following the protocol by Wilson³⁴ with slight modifications. The test mixture contained GSH (1 mM), ethylene diamine tetra acetate (EDTA, 1 mM), glutathione reductase (GR) (1.3 unit·mL⁻¹), and β -nicotinamide adenine dinucleotide phosphate (NADPH, 0.2 mM) in potassium phosphate buffer (100 mM), pH 7.5. Catalysts (20 μ M) were added to the test mixture at 21 °C and the reaction was initiated by addition of H₂O₂ (0.8 mM). Initial reaction rates were based on the consumption of NADPH as assessed by UV-spectroscopy at 340 nm. The initial reduction rates were triplicated and calculated from the first 10 seconds of reaction by using 6.22 mM⁻¹cm⁻¹ as the extinction coefficient for NADPH. GPx-data reported in Table 2 are means \pm SD.

Biological Studies. Blood buffy coats were diluted in a 1:1 ratio with 1 x phosphate buffered saline (PBS) and mononuclear cells were segregated using Ficoll-Paque plus density gradient centrifugation. Briefly, the diluted blood was gently placed on top of Ficoll-Paque and then centrifuged at 400 g for 30 minutes. After removing the plasma layer, the mononuclear (MNC) layer was collected, washed with PBS, and then spun at 100 g for 15 min. This washing procedure was repeated for a total of 3 times. Total cell number was counted using a hemocytometer and trypan blue exclusion method. The blood pellet was then suspended in 3% dextran/0.9% saline for 20 min. The supernatant was then collected and the cell pellet collected after centrifugation at 250 g for 10 min. To remove erythrocyte, the pellet was subjected to 0.2% saline solution for 20 s and then an equal volume of 1.6% saline was added. Then the cells were spun at 250 g for 10 min and the pellet was suspended in PBS.

Chemiluminescence Assay. To measure release of ROS from monocytes, a luminol amplified chemiluminescence assay was conducted. All measurements were performed in white 96 well plates at 37°C in 1 x PBS with 50 mM of luminol, 0.1M NaOH, 2 μ g/mL of horse radish peroxidase, different concentrations of ebselen, **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b** and Trolox initially diluted in DMSO. Approximately 2 x 10⁵ cells were plated in each well and then stimulated to generate ROS using 500 nM of 1 μ M phorbol myristate acetate (PMA). Luminescence readings were taken using a multi-plate reader every 2 min for 2 h. Total ROS was quantified by determining the area under the chemiluminescence kinetic curve. Experiments were repeated 4-5 times with n = 3 per group per

experiment. For statistical analysis, ANOVA was performed with Scheffe post-hoc tests. P values below 0.05 were considered significant.

Cell proliferation and toxicity assay. MC3T3 (plating density: 5×10^3 and 20×10^3 per well respectively in a 96 well plate) was let adhered after plating for 24 h in α -MEM supplemented with 10% FBS and 1% pen/strep. Then the cells were exposed to antioxidants **3**, **6b-c**, **7a-b**, **7d**, **8b**, **8d** and **11b** (25 μ M) for 24 h, washed and then fed with fresh α -MEM every other day. To assess cell viability, the Alamar Blue assay was conducted on day 1 and 3 after treatment. Briefly, cells were washed with 1 x PBS and then incubated with Alamar Blue (1:20 dilution in phenol red free α -MEM for 1.5 h and then fluorescence was read with a plate reader at 560 nm excitation and 590 nm emissions. Experiments were repeated twice with $n = 6$ for each group.

Mechanistic studies. A solution of H_2O_2 (3.7 μ L, 0.033 mmol) was added to an NMR tube containing diselenide **8b** (20 mg, 0.033 mmol) in 400 μ L of $DMSO-d_6$ and the ^{77}Se NMR spectrum was recorded after 30-60 min. Then another equivalent of H_2O_2 was added and the ^{77}Se NMR spectrum recorded again. Several portions (1+2 equivalents) of GSH (10 mg, 0.033 mmol) in 100 μ L of H_2O were then syringed into the NMR tube. After every new addition of thiol the ^{77}Se NMR spectrum was recorded.

ASSOCIATED CONTENT

Supporting Information

1H , ^{13}C and ^{77}Se NMR data for all new compounds prepared. Results from kinetic studies of compounds prepared and coordinates of optimized geometries. X-ray crystallographic data (CIF files). This material is available free of charge via the Internet at <http://pubs.acs.org>

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Notes

The authors declare no competing financial interest

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